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TITLE: Inflammation Modulatory Protein TSG-6 for Chemical Injuries to the Cornea

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Temple, Texas 76504-7451

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT This period we tested the efficacy of intravenous(IV) TSG-6 alone, and in combination with topical application. We performed further experiments with anterior chamber(AC) TSG-6 and high dose IV application, and reported our results at the American Society of Cataract and Refractive Surgery Symposium in Los Angeles in May 2017. We found that IV and AC application reduced selected inflammatory markers at day 1, but not at later time points to day 7, and with no reduction in corneal opacity at any time point. High dose IV TSG-6 or IV TSG-6 in combination with topical TSG-6 did not result in biochemical or clinical benefit, and delayed application of TSG-6 four hours after injury was ineffective. Current experiments are investigating the use of exosomes that contain TSG-6 to determine efficacy in the treatment of corneal alkali burns, and we have submitted an amendment pending approval to investigate the use of TSG-6 containing exosomes in the treatment of nitrogen mustard injury to the eye.					
15. SUBJECT TERMS cornea, alkali injury, rat, inflammation, chemical burn, TSG-6					
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1. INTRODUCTION:

This project has studied the treatment of chemical injury of the cornea with a natural anti-inflammatory protein, TSG-6, which has a novel mechanism of action. Chemical injuries of the eye are difficult to treat, and may lead to severe debilitation or blindness with few patient treatment options. The wartime threat for chemical injury to the eye is evidenced by the thousands of soldiers injured with mustard gas in the Iran-Iraq war. The mechanism of injury after chemical ocular injury includes inflammation secondary to trauma, and may be alleviated with anti-inflammatory agents like TSG-6. TSG-6 works by inhibiting inflammation at the earliest stage, and is effective in the treatment of mild chemical injuries caused by alcohol and mechanical scraping in a mouse model. This study was designed to determine if TSG-6 would be effective with more severe chemical alkali injuries of the cornea, and which would more closely mimic injuries from mustard gas or other severe caustic agents. We investigated the biochemical and clinical effects of topical, anterior chamber, and IV TSG-6 in a rat model of cornea chemical injury to determine the efficacy of TSG-6 in chemical injury to the eye.

2. KEYWORDS:

Cornea, alkali injury, eye trauma, chemical injury, anti-inflammatory protein, stem cells, TSG-6, regeneration

3. ACCOMPLISHMENTS:

a. Project Goals

The specific goals of the project for the third year of the project per the SOW included:

- 1) Specific Aim 3 Subtask 1: Determine the efficacy and dose response of IV TSG-6
- 2) Specific Aim 3 Subtask 2: Determine the therapeutic window of IV TSG-6 administration
- 3) Specific Aim 3 Subtask 3: Test for synergistic effect of topical and intravenous administration of TSG-6

3. ACCOMPLISHMENTS, ctd

4) Specific Aim 3 Subtask 4: Maximal rescued animals

b. Goals Achieved

1) Specific Aim 3 Subtask 1: Determine the efficacy and dose response of IV TSG-6

Rat corneas were exposed to 0.5N NaOH for 30 seconds using filter paper soaked with NaOH, and then the corneas were thoroughly rinsed with 50cc of BSS. The corneas were photographed for clinical scoring daily for seven days, and the corneas were collected for cytokine PCR assay for RNA at days one and seven post injury. TSG-6 was delivered through tail vein injection immediately after injury. Experiments this reporting period indicate that IV TSG-6 resulted in a modest but statistically significant reduction in inflammatory signaling for IL1-B and neutrophil elastase at day 1 for the 0.5N injury level, but with no significant benefit with respect to corneal clarity at any time point post injury. Experiments with high dose IV TSG-6 did not demonstrate efficacy with respect to inflammatory markers or corneal clarity, which indicates that there is no additional benefit at higher TSG-6 dose levels. The modest reduction in inflammatory signaling noted at day 1 for IV TSG-6 was mirrored in additional experiments performed with anterior chamber (AC) TSG-6 application, and these data were reported at the American Society for Cataract and Refractive Surgery Annual Symposium in Los Angeles May 5-9, 2017. **Appendix 1, p10-27.**

2) Specific Aim 3 Subtask 2: To determine the therapeutic window for IV TSG-6

We investigated the time window of application for IV TSG-6 since others have reported that TSG-6 must be administered rapidly after injury to be effective. We found that while IV TSG-6 given at the time of injury results in a modest reduction in inflammatory markers, TSG-6 given 4 hours after injury does not result in the same reduction. Our results confirm the work of others which indicates that TSG-6 must be given as close to the time of injury as possible to be effective. **Appendix 2, p28-31.**

3) Specific Aim 3 Subtask 3: Test for synergistic effect of topical and intravenous administration of TSG-6

We investigated for synergistic effect of IV and topical TSG-6 applied at the time of injury, and found no synergistic effect with respect to inflammatory markers or corneal clarity, which further confirms the lack of efficacy of topical TSG-6 after chemical injury to the eye. **Appendix 3, p32-34.**

3. ACCOMPLISHMENTS, ctd

4) Specific Aim 3 Subtask 4: Maximal rescued

Experiments with TSG-6 did not result in clinical benefit at any time point, and did not justify further investigation into maximal rescued rats with maximal effective dose experiments.

c. Training Opportunities and Professional Development

Nothing to report

d. Dissemination of Findings and Results

1) Manuscript published October 2017: **Comprehensive Profiling of Alkali Injuries to the Cornea. Curr Eye Res 2017 Oct;42(10):1348-1357.**

2) Paper presented May 2017 at the American Society of Cataract and Refractive Surgery: **The Efficacy of TSG-6 for Acute Corneal Alkali Injury in a Rat Model. (Appendix 1)**

3) Paper submitted for presentation, acceptance pending, for the American Society of Cataract and Refractive Surgery, Washington DC, April 2018. **Appendix 4, p35.**

4) Manuscript in preparation: The Efficacy of TSG-6 for Acute Corneal Alkali Injuries in a Rat Model

e. Plans for Next Reporting Period

1) We have been approved for a no cost extension on our study through March 2018, and plan to continue experiments with extracellular exosomes from mesenchymal stem cells produced under stress and which contain TSG-6. We also plan to test the effectiveness of exosomes that contain TSG-6 in the treatment of ocular injury caused by nitrogen mustard application.

2) We plan to complete our manuscript which will discuss our results to date on the efficacy of TSG-6 for corneal alkali injury in our rat model.

4. IMPACT

a. Principal Discipline

The results of this work for this report significantly add to the body of data which have previously demonstrated the beneficial anti-inflammatory effects of TSG-6 in other models of tissue injury or human disease. It is apparent from our data that the severity of tissue damage as which occurs in alkali ocular injury may limit clinical outcomes in models of TSG-6 treatment,

and is an important contribution to the research community. Our data points to new physiologic methods of TSG-6 delivery to the site of injury with exosomes, and to less severe types of ocular chemical injury with secondary inflammatory responses, such as occur with nitrogen mustard injury.

b. Other Disciplines

The results of this work, albeit with lack of demonstrated efficacy of TSG-6 to date for corneal alkali injury, will help investigators in the field of Regenerative Medicine focus on alternate methods of delivery, and to demonstrate that while TSG-6 has been shown to be effective in a model of mild chemical corneal injury induced by ethanol, it is insufficient to benefit more severe ocular alkali chemical injuries. This work also sets the stage for further investigation into the possible use of TSG-6 contained within exosomes to treat ocular injury sustained by alkylating agents such as nitrogen mustard, which do not have the depth of ocular penetration as sodium hydroxide or other alkali agents.

c. Technology Transfer

Nothing to report

d. Impact on Society

Nothing to report

5. CHANGES/PROBLEMS

a. Changes

1) We have been granted a no cost extension to continue our work to study the efficacy of TSG-6 delivered through exosomes in corneal alkali injury, and to perform initial studies of the use of TSG-6 in the treatment of corneal injury caused by nitrogen mustard, an alkylating agent present in modern day battlefields.

b. Actual/Anticipated Problems

1) None to report

c. Changes in Expenditures

1) None to report

d. Changes in Animal Use

1) None to report

6. PRODUCTS

a. Publications/papers/presentations

1) Manuscript published October 2017: **Comprehensive Profiling of Alkali Injuries to the Cornea.** *Curr Eye Res* 2017 Oct;42(10):1348-1357.

2) Paper presentation, **The Efficacy of TSG-6 for Acute Corneal Alkali Injuries in a Rat Model.** American Society of Cataract and Refractive Surgery Annual Symposium, Los Angeles, California, May 5-9, 2017.

b. Websites

1) Central Texas Veterans Research Foundation <http://www.ctvrf.org/research-programs>

c. Technologies/Techniques

Nothing to report

d. Inventions/Patents/Licenses

Nothing to report

7. PARTICIPANTS/COLLABORATING ORGANIZATIONS

a. Individuals

1) Name:	Hosoon Choi, PhD
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	35
Contribution to Project:	Dr. Choi has performed rat surgery to treat corneal alkali injury, performed chemokine and cytokine assays, and protein production and purification

2) Name:	Casie Phillips
Project Role:	Animal Technician, research assistant
Researcher Identifier (e.g. ORCID ID):	NA

Nearest person month worked: 34

Contribution to Project: Casie has assisted Dr. Choi in animal surgery, performs postoperative care, assists with laboratory techniques, prepares and stains histology specimens

3) Name: Samuel Fulcher, MD

Project Role: PI

Researcher Identifier (e.g. ORCID ID): NA

Nearest person month worked: 36

Contribution to Project: Dr. Fulcher performs the duties of the PI, and scores the clinical injuries by grading photographs of the corneal opacity

b. Collaborating Institutions

1) Texas A&M Institute for Regenerative Medicine, Temple, Texas. Dr. Darwin Prockop and his team have served as unpaid consultants.

2) Central Texas Veterans Research Foundation, Temple, Texas. CTVRF is the sponsoring agency.

3) Central Texas Veterans Health Care System, Temple, Texas. CTVHCS is the employer of Dr. Fulcher, and has allotted time for research activities, and has supported the project with laboratory space, supplies, and equipment.

4) Baylor Scott and White Health Care, Temple, Texas. BSW is the sponsor of the IACUC, and operates and maintains the vivarium where experiments are performed, and employs the supervising veterinarian who oversees all animal research projects.

8. SPECIAL REPORTING REQUIREMENTS

a. No Collaborative Awards to report

b. Quad Chart, Appendix 5, p36-37.

9. APPENDICES, SEE APPENDICES A1-A5

Appendix 1

The Efficacy of TSG-6 for Acute Corneal Alkali Injury in a Rat Model

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Financial Disclosure

No authors have any financial interests to disclose

IACUC approved protocol

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Chemical Injuries of the Cornea

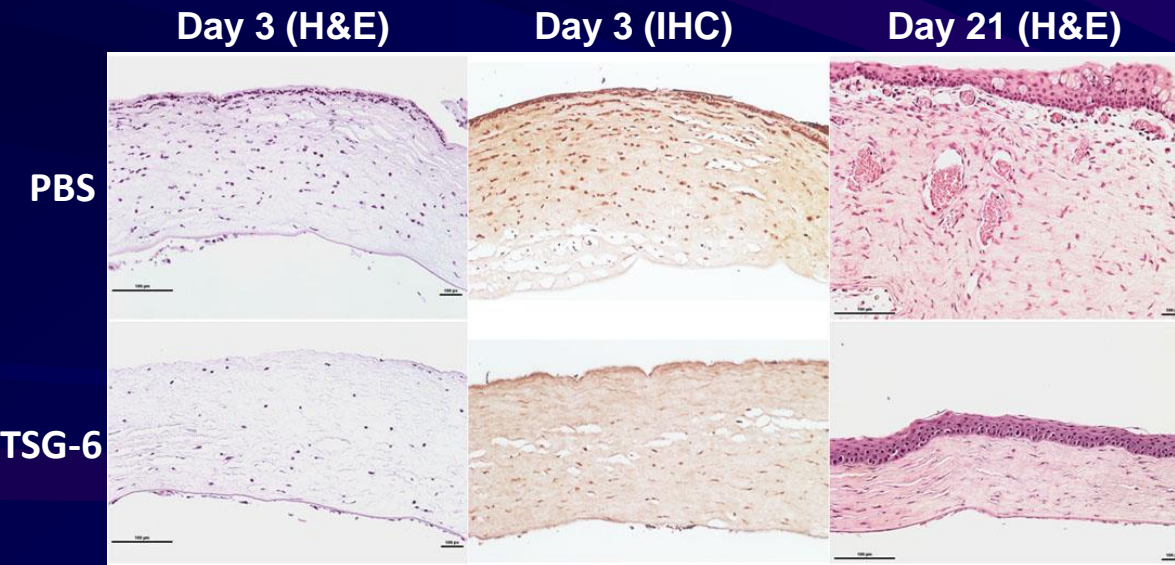
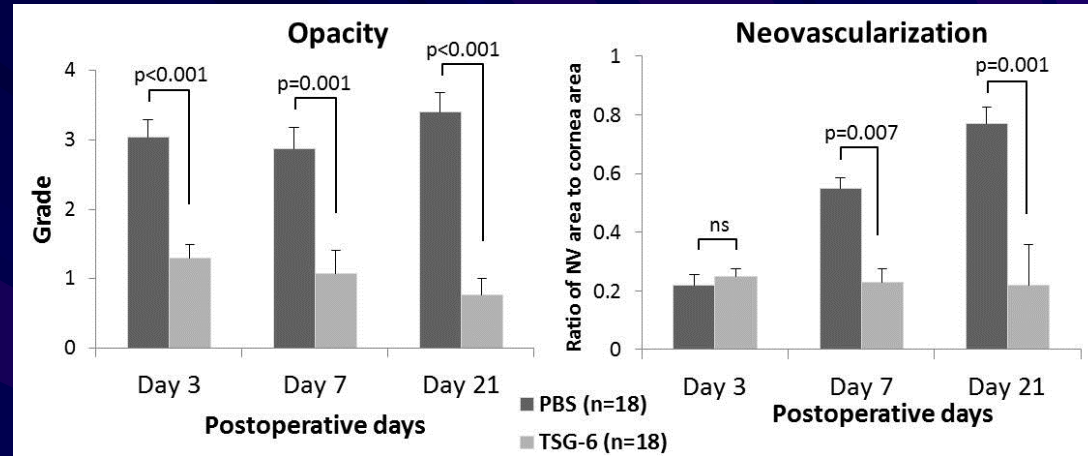
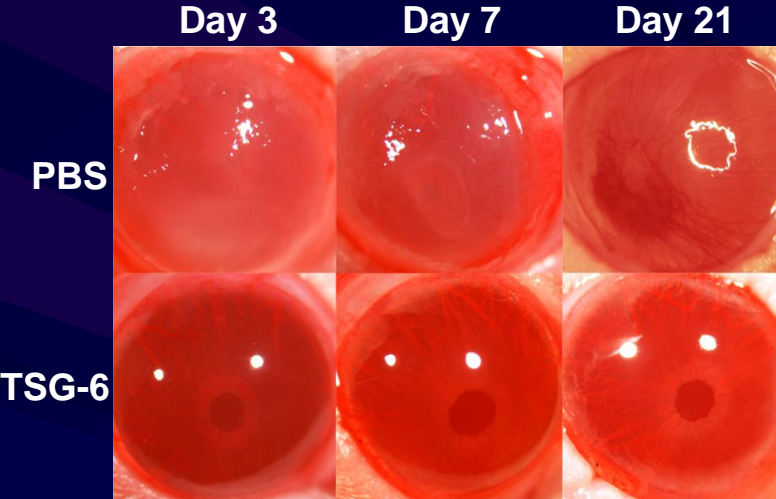
Tissue Damage:

- ... Direct chemical injury
- ... Compounded by inflammation
- ... Steroids and NSAIDS may amplify

A Potential Solution:

- ... The protein **"TSG-6"**
 - ... TNF-stimulated gene 6 protein
 - ... modulator of inflammation
 - ... novel mode of action

MOUSE ETHANOL/SCRAPING MODEL



Anterior chamber (AC) TSG-6

Reduced opacity
Reduced neovascularization
Decreased invasion of inflammatory cells

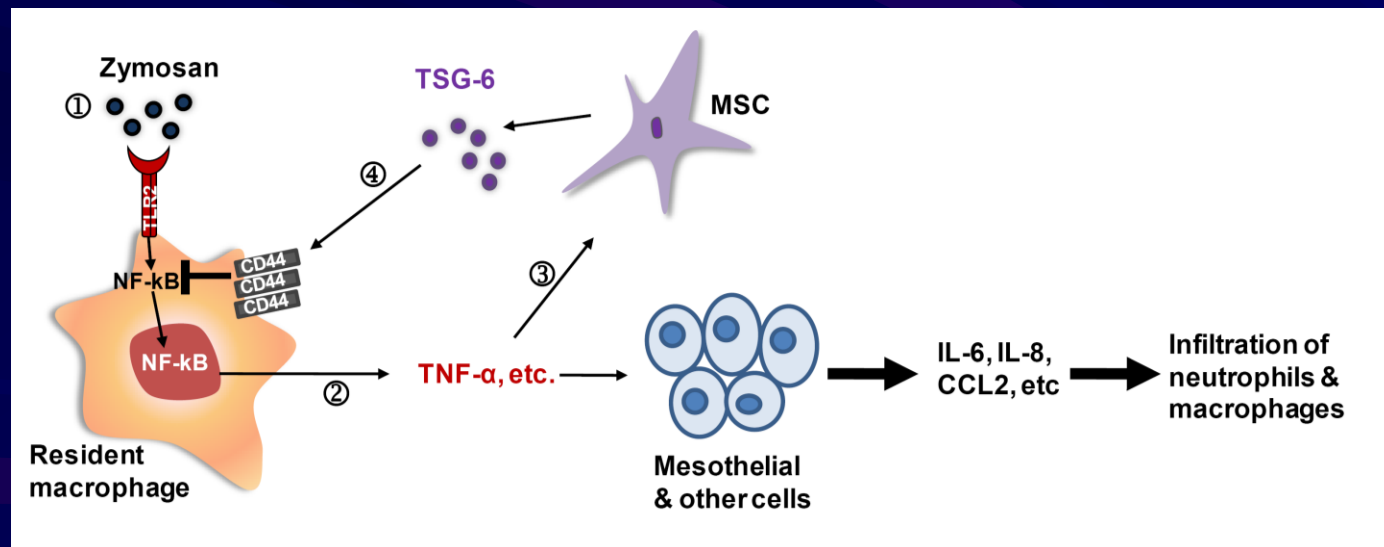
Oh et al., PNAS 2010

Unique Mechanism of TSG-6

... A natural negative feedback loop for inflammation

...Released by Mesenchymal Stem Cells (MSCs)
blocks macrophage NF- κ B pathway via CD44 receptor
“first responders” to tissue injury

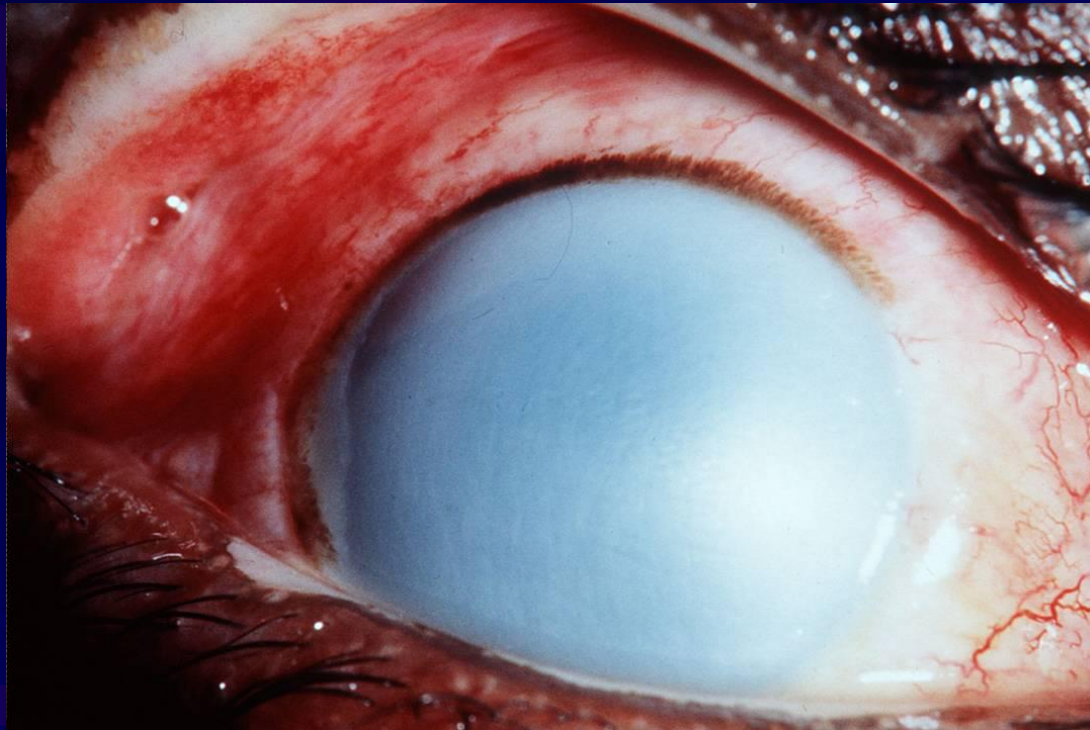
...no known toxic effects in eye or other tissues



A More Difficult Injury: **Corneal Alkali Burn**

...alkali penetrates rapidly and deeply

...requires prolonged irrigation to reduce pH



Experimental Design

Lewis Rats (8 per group)

0.5N NaOH, 4 mm paper, 30 sec exposure, extensive rinse

TSG-6: Topical qts tid 0.75 ug/ml

AC 4 ug

IV 200 ug

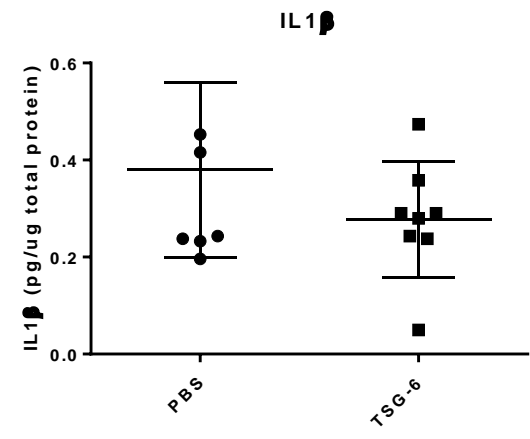
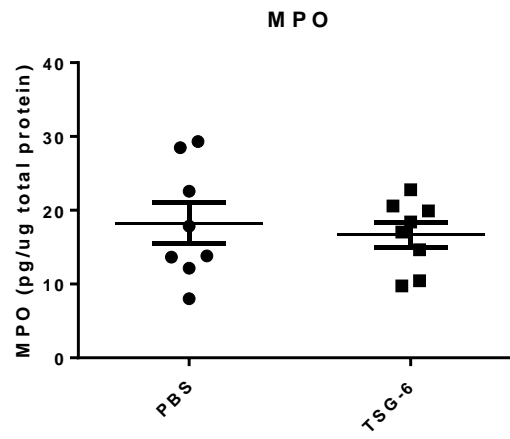
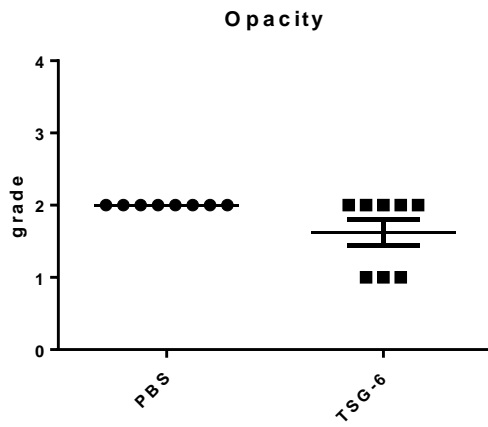
PBS controls

Inflammatory marker assays

Opacity Grading

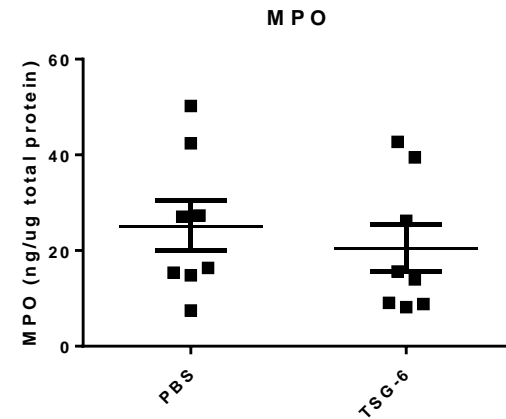
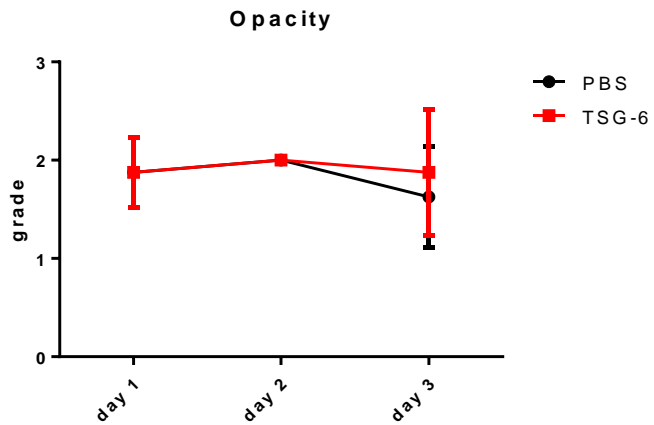
TOPICAL TSG-6

Day 1



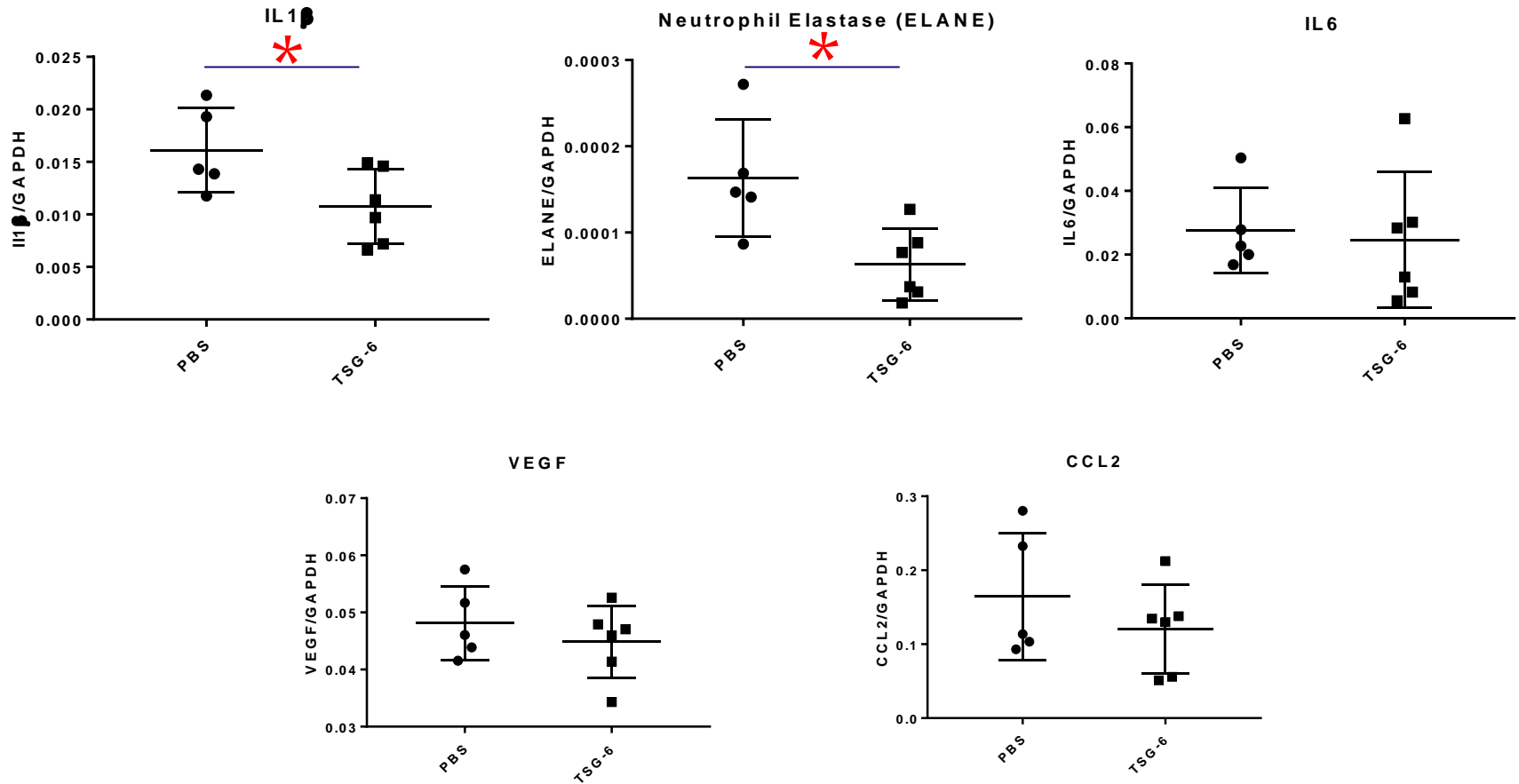
TOPICAL TSG-6

Day 3



IV TSG-6

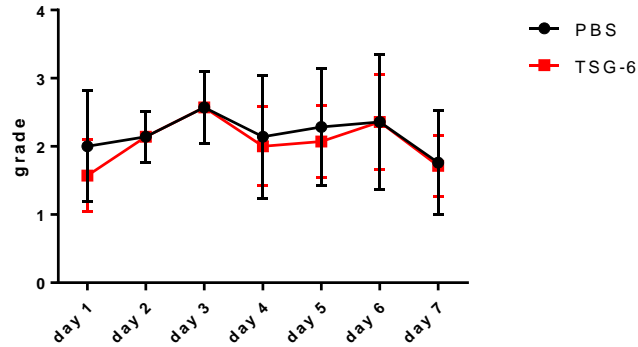
Day 1



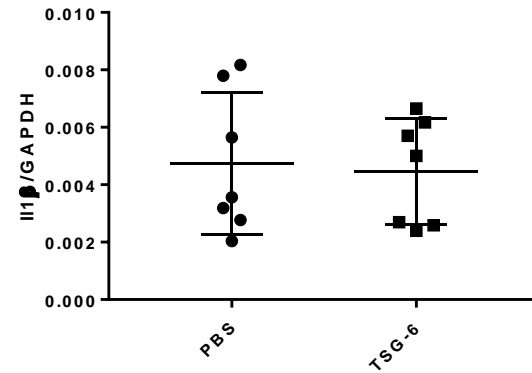
IV TSG-6

Day 7

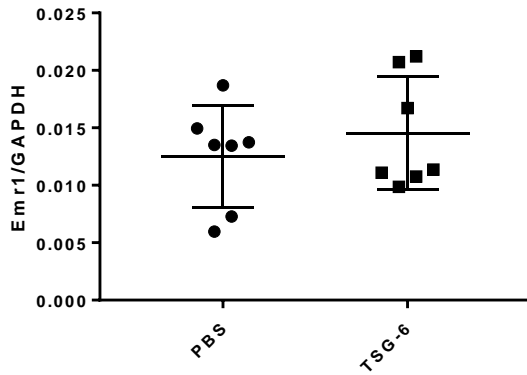
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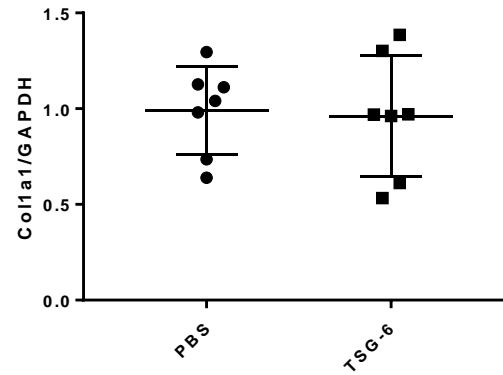
IL1 β



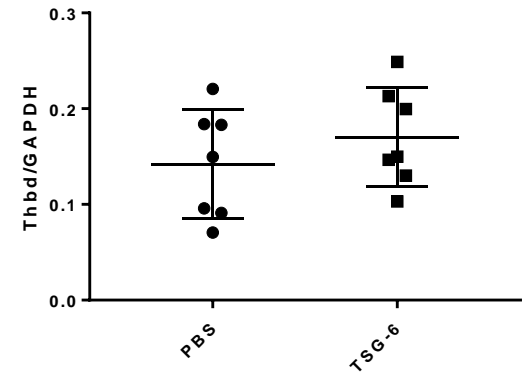
Emr1



Col1a1



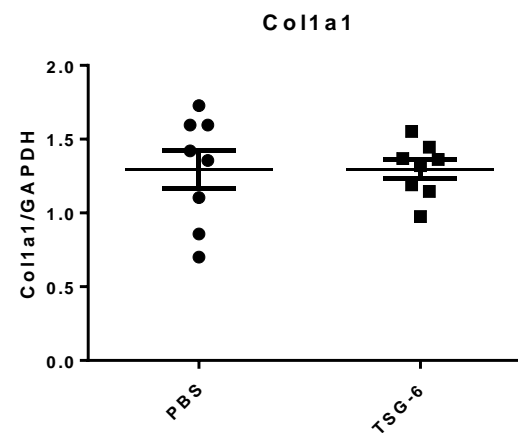
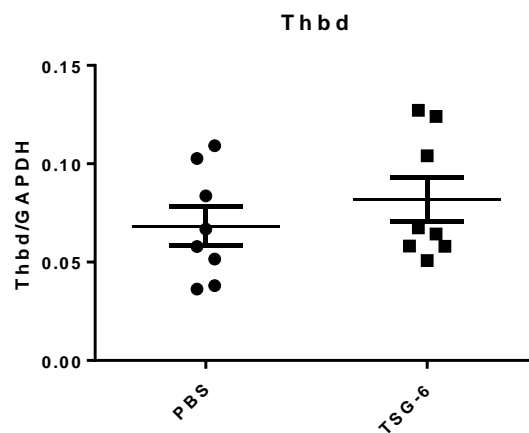
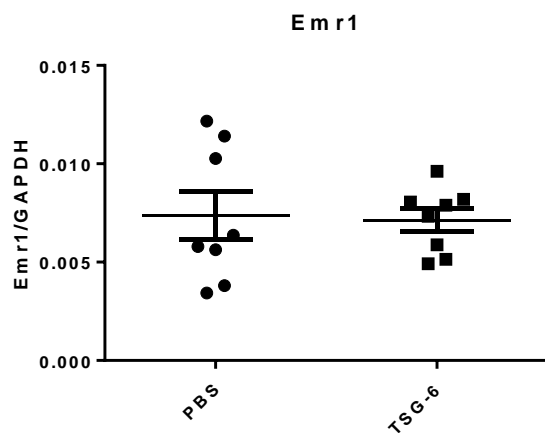
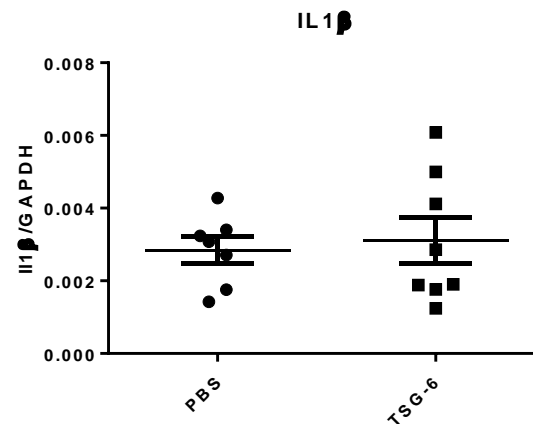
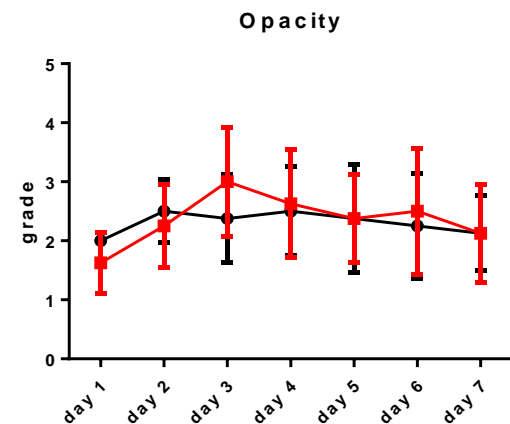
Thbd



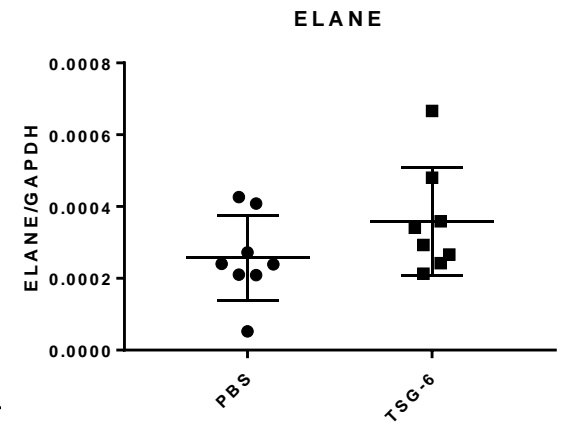
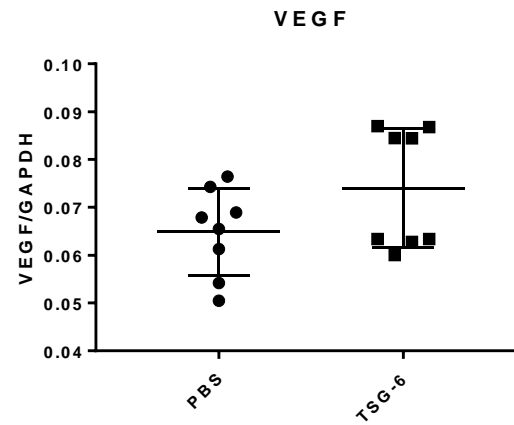
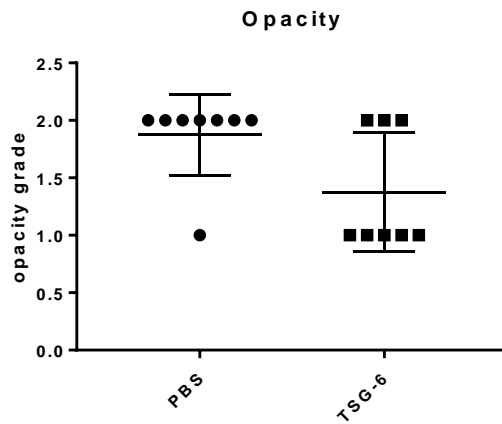
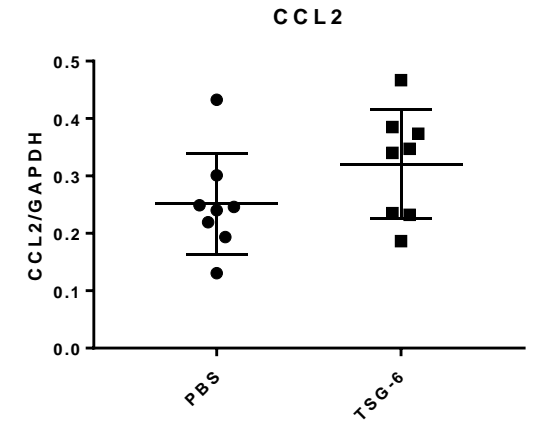
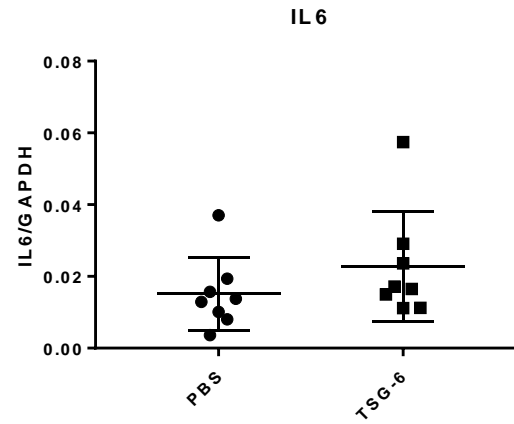
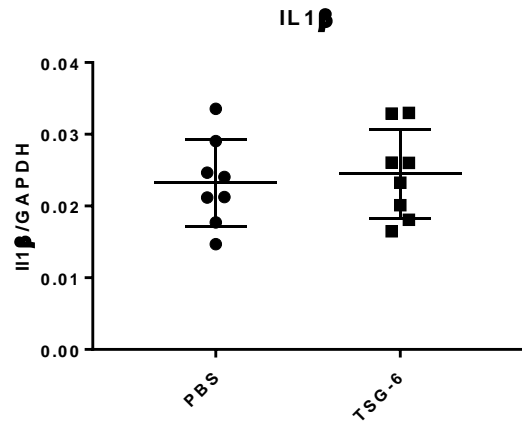
MULTI DOSE IV TSG-6

0, 6, 24 HOURS

DAY 7

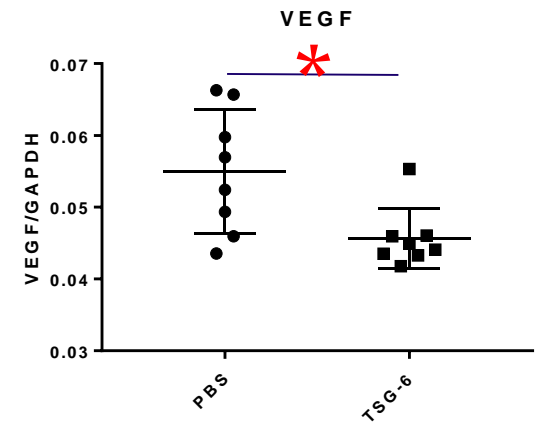
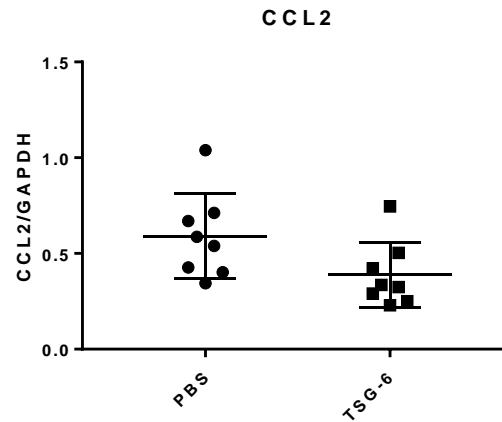
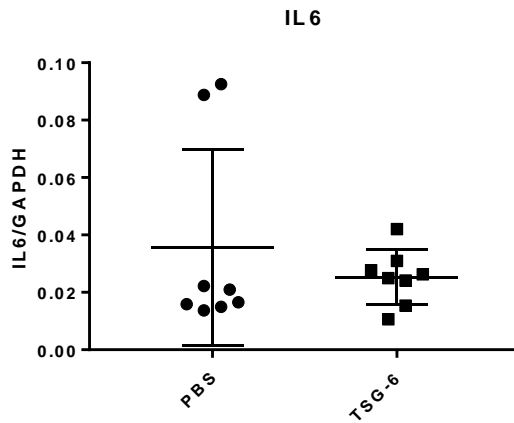
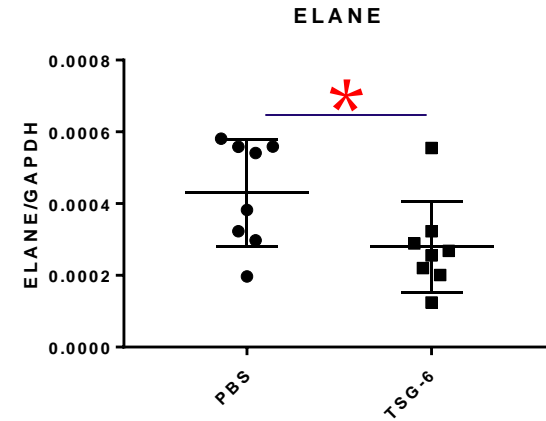
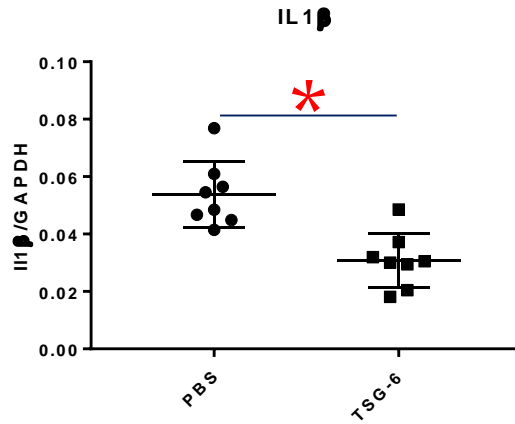


HIGH DOSE IV TSG-6 400 ug DAY 1



AC TSG-6

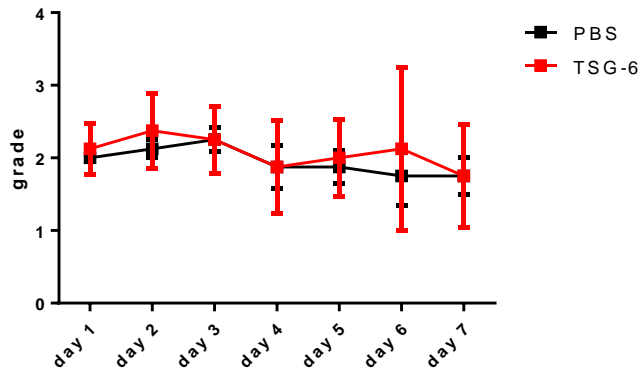
Day 1



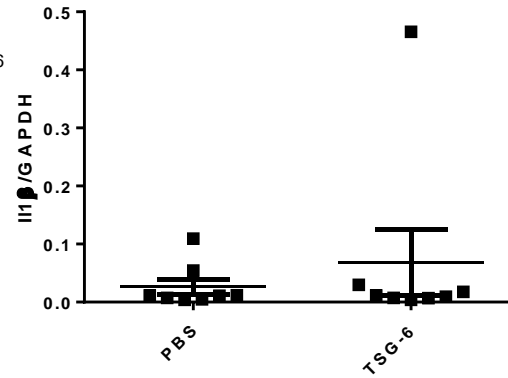
AC TSG-6

Day 7

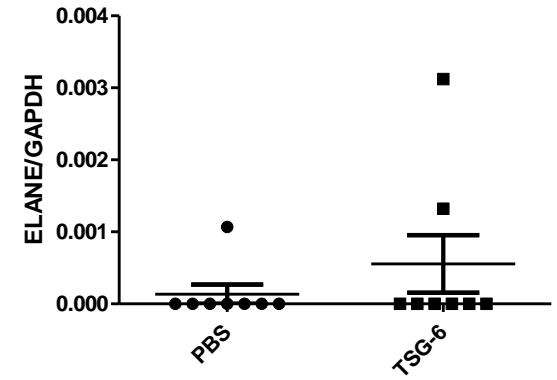
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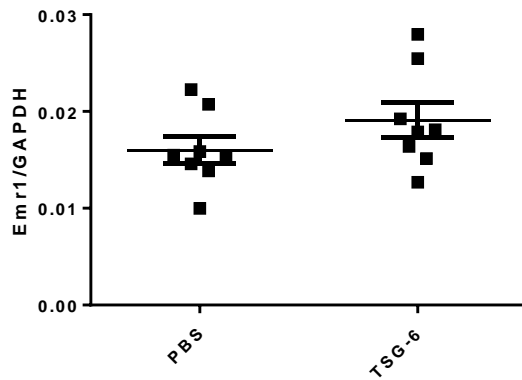
IL1 β



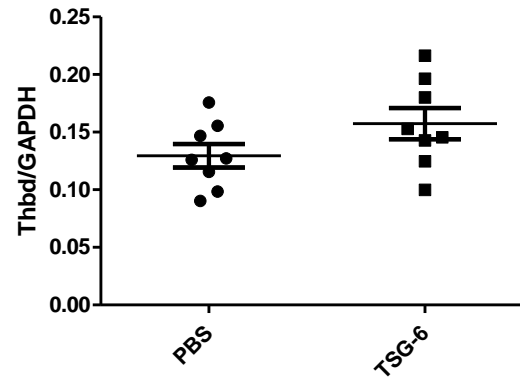
ELANE



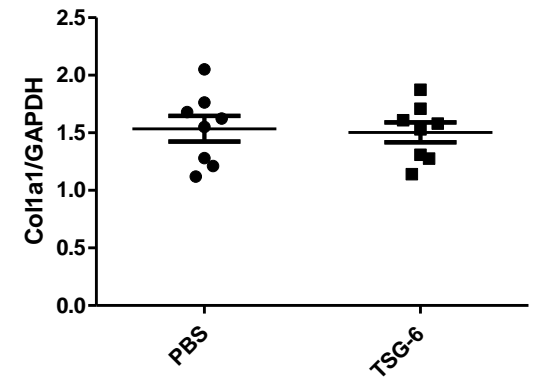
Emr1



Thbd



Col1a1



RESULTS

AC & IV TSG-6 reduce inflammatory markers at Day 1

BUT (a) Topical TSG-6 demonstrated no effect

(b) Corneal opacity was not reduced

Conclusions and Future Studies

Rat model for corneal alkali injury

...**AC/IV TSG-6 reduced inflammation Day 1**

...Topical TSG-6 ineffective

...No benefit yet on corneal opacity

TSG-6 holds promise in chemical corneal injury

...A natural protein

...Unique mechanism of action

...**High dose**, multiple dose

...**Possible synergies** with new anti-apoptotic peptides

Appendix 2

IV Injection Delayed Treatment Day 1

NaOH 0.5 N (30 sec); 50 mL saline flush + 50 mL saline rinse (15 minutes)

- Intravenous injection (4 hour after injury; delayed treatment):

200 μ g / rat

- Rats:

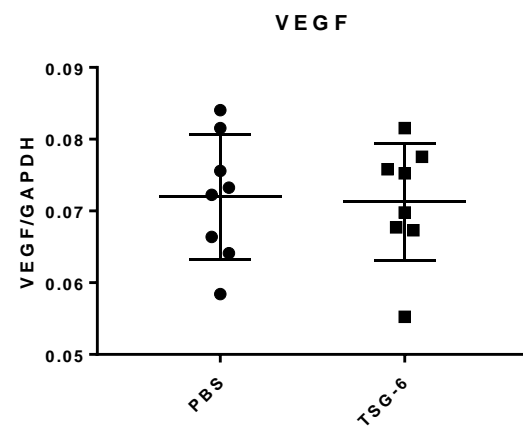
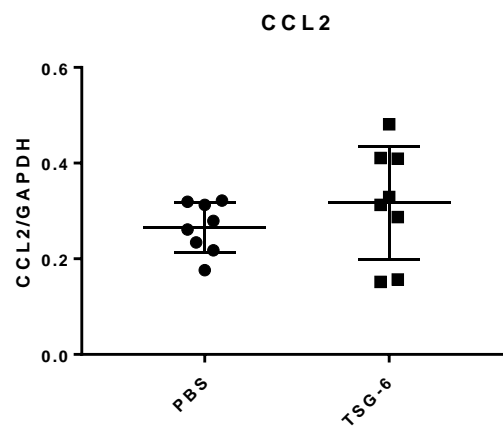
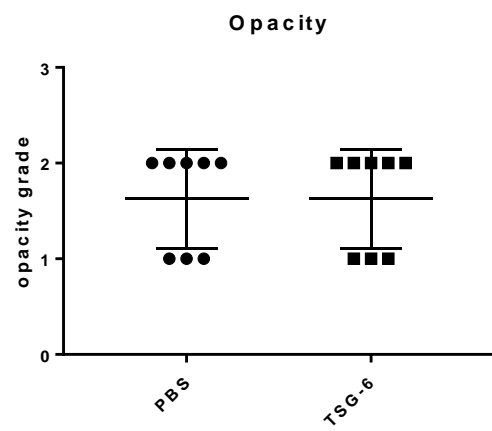
8 rats / PBS

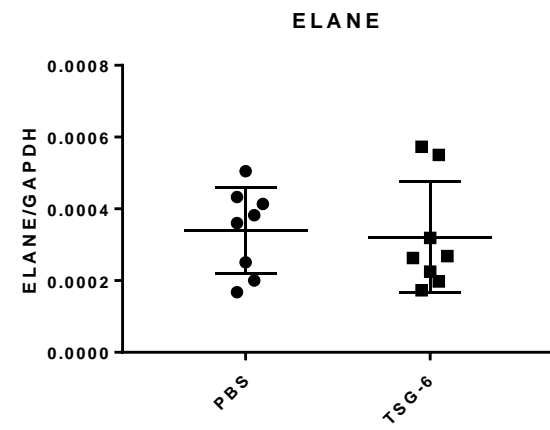
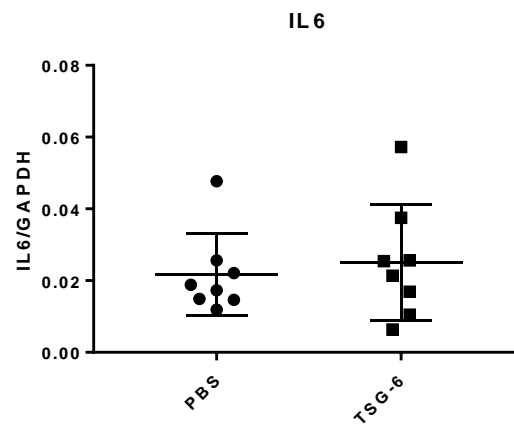
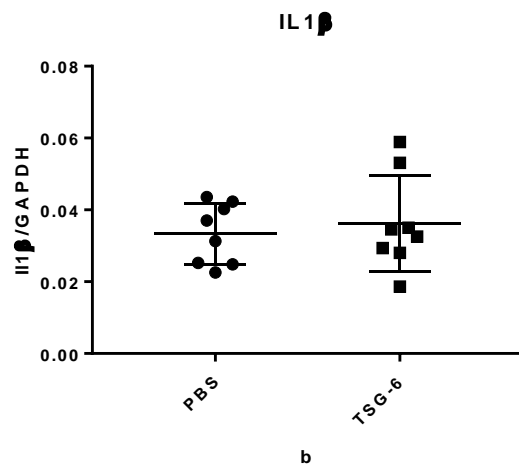
8 rats / TSG-6 = 16 Lewis rats total

- Harvest day / number of animals:

Day 1 harvest / 16 rats







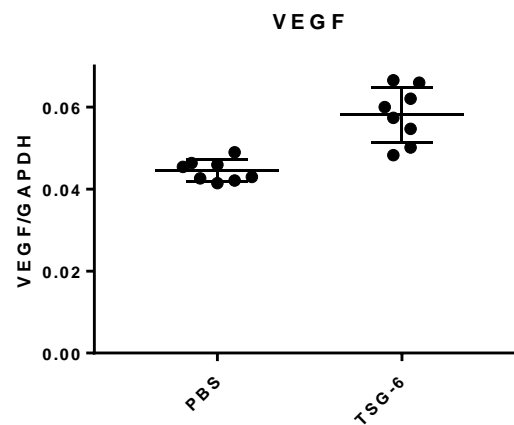
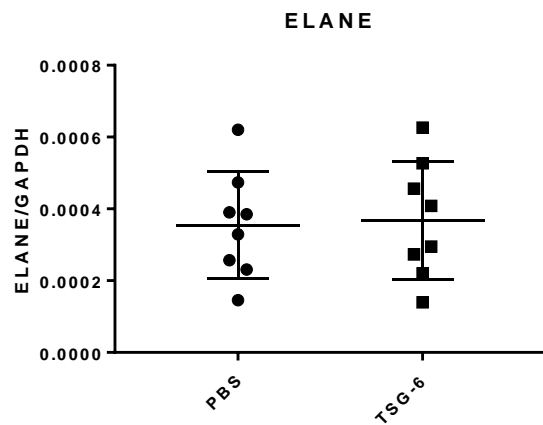
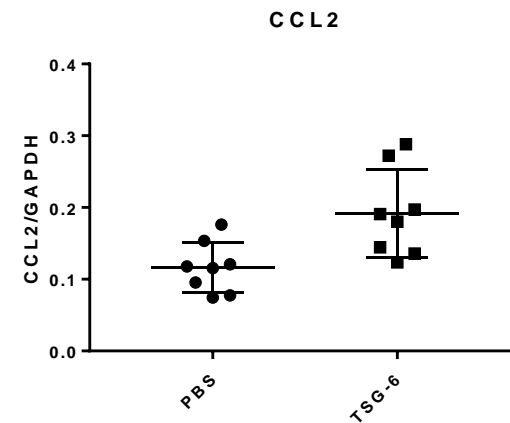
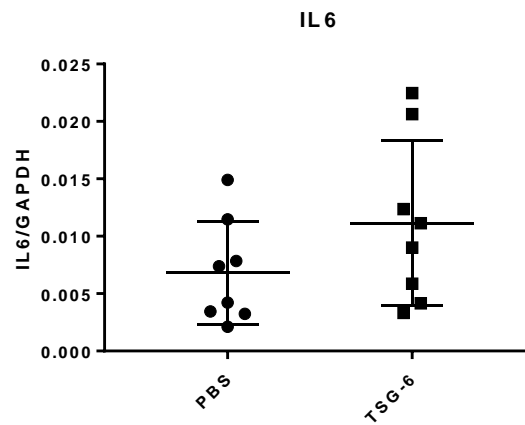
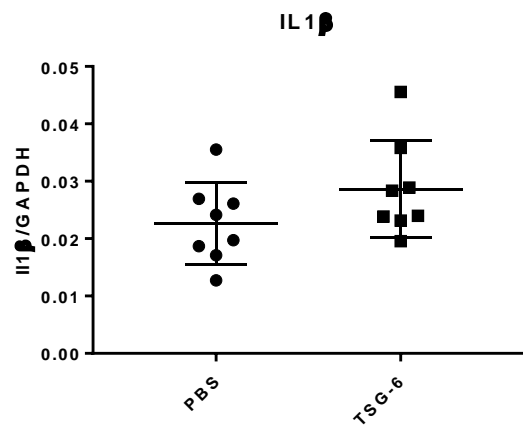
Appendix 3

IV Injection + topical

NaOH 0.5 N (30 sec); 50 mL saline flush + 50 mL saline rinse (15 minutes)

- Intravenous injection :
200 μg / rat
- Topical treatment:
5 μl / drop
1 drop, 3x/day
- Rats:
8 rats / PBS
8 rats / TSG-6 = 16 Lewis rats total
- Harvest day / number of animals:
Day 1 harvest / 16 rats





Appendix 4

Title:

The Efficacy of the Anti-Inflammatory Protein TSG-6 for Acute Corneal Alkali Injury in a Rat Model

Submitter's E-mail Address:

samuel.fulcher@va.gov

Category:

Non-Clinical

Agreements:

I understand all posters are virtual, not printed posters.

I agree to the copyright assignment terms.

I understand if my submission is accepted, that I am required to register and pay an attendee fee for the Symposium and Congress.

Keywords:

Alkali Burn, Cornea and Inflammation

Purpose:

We determined the efficacy and optimal time course of application of the promising inflammation modulatory protein TSG-6 in the treatment of corneal alkali injury in a rat model via topical drops, anterior chamber injection, or intravenous injection given singly or with multiple doses.

Methods:

Rat corneas in each group were exposed to 0.5N NaOH soaked filter paper and thoroughly rinsed. TSG-6 was applied immediately after injury either topically, via anterior chamber (AC) or intravenous (IV) injection, and at delayed time points after injury in an IV group, and were matched with saline treated controls. The corneas were assayed for inflammatory markers on days 1 and 7, and were graded for opacity over a 7 day time course in a blinded manner by two corneal specialists. Additional experiments evaluated multi-dose application of high dose IV TSG-6 within 24 hours of injury. Results from treatment and control groups were analyzed and compared.

Results:

A significant decline ($p < .05$) was found at day 1 for inflammation markers IL1b and ELANE for IV TSG-6 given immediately post injury, but no significant differences were noted for IV TSG-6 applied 4 hours later. A significant decline in IL1b and VEGF was found at day 1 for AC TSG-6 applied immediately post injury, but topical TSG-6 resulted in no difference in inflammatory signaling. No differences in inflammatory signals were found for all routes of TSG-6 delivery compared to controls by day 7, and there was no difference in corneal clarity at all time points between treatment and control groups. Multi-dose IV TSG-6 did not improve inflammatory signaling or corneal clarity by day 7.

Conclusion:

In this rat model of corneal alkali injury, IV or AC application of TSG-6 given immediately post injury reduces inflammatory signals at day 1 but not day 7, and does not improve corneal clarity by day 7, while topical or delayed TSG-6 is ineffective. Multi-dose IV TSG-6 did not improve inflammatory signals or corneal clarity by day 7 in this model.

Samuel F.A. Fulcher, MD (Presenting Author); Casie Phillips; Roxanne L. Reger, MS; Joo Youn Oh, MD, PhD; Hosoon Choi, PhD

Appendix 5

Inflammation Modulatory Protein TSG-6 for Chemical Injuries to the Cornea

MR130174

W81XWH-14-1-0495



PI: Samuel F.A. Fulcher MD

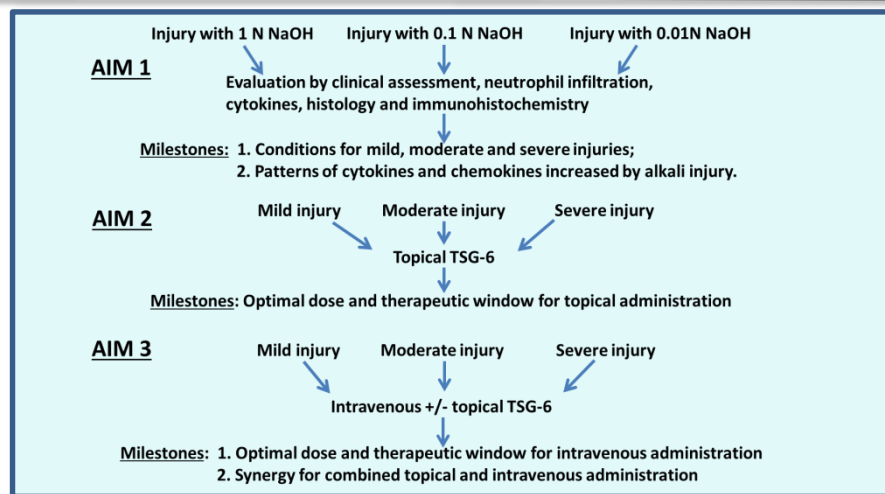
Org: Central Texas Veterans Foundation Award Amount: \$985,149

Study/Product Aim(s)

- **Aim 1.** Determine the timing and patterns of inflammation and other cellular and molecular changes in response to the severity of alkali injury. The data will allow us to select the optimal conditions for evaluating the effectiveness of TSG-6 therapy in Aims 2 and 3.
- **Aim 2.** Establish the optimal dose and the time window for effective topical and anterior chamber administration of TSG-6 therapy as a function of the severity of the alkali injury.
- **Aim 3.** Establish the optimal dose and the time window for effective intravenous administration of TSG-6 as a function of the severity of the alkali injury as well as combined topical and IV administration.

Approach

Expose the corneas of rats to varying concentrations of alkali. Assays of inflammatory markers and clinical grading of injury and healing will be used to assess effectiveness of treatment. The results will establish the limits under which the limbal epithelial stem cells can still be rescued by modulating inflammation with TSG-6.



Outline of Proposed Project

Timeline and Cost

Activities	CY	14	15	16	17
Aim 1. Establish appropriate conditions for testing TSG-6.					
Aim 2. Optimal dose and time window for topical, AC TSG-6.					
Aim 3. Optimal dose and time window for intravenous and topical + IV TSG-6.					
Estimated Budget (\$K)		\$298	\$356	\$331	\$985

Goals/Milestones (Example)

CY14 Goal – Establish the appropriate conditions for testing TSG-6

- ☒ Determine timing and patterns of cellular and cytokine inflammatory responses as a function of alkali injury severity.

CY15 Goals – Optimize treatment parameters for topical TSG-6.

- ☒ Optimize topical dose of TSG-6
- ☒ Determine time window for topical therapy

CY16 Goal – Optimize treatment parameters for intravenous TSG-6.

- ☒ Optimize intravenous dose of TSG-6
- ☒ Determine time window for intravenous therapy
- ☒ Determine synergistic effects of combined topical and intravenous TSG-6.

Comments/Challenges/Issues/Concerns

- Aims 1,2,3 are completed.

Budget Expenditure to Date

Projected Expenditure: \$985,184

Actual Expenditure: \$896,297 (as of 10/5/2017)

Updated: (10/1/2017)